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COMMUNICATIONS

ARGEMONE OIL, SANGUINARINE, AND EPIDEMIC-DROPSY GLAUCOMA*†

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Argemone mexicana Linn. (Linnaeus, 1753; Tournefort, 1694), is a herb of the *Papaveraceae* (poppy family) indigenous to the West Indies (Fig. 1).

The study of this herb and its active principles is important, first to circumvent the serious epidemics of dropsy and glaucoma resulting from the unintentional ingestion of its seed oil in tropical countries; secondly to find a method of producing in laboratory animals conditions similar to, if not identical with, glaucoma; and thirdly to examine the possibility that other similar plants, by indirect ingestion, may play an insidious role in the causation of primary endemic glaucoma.

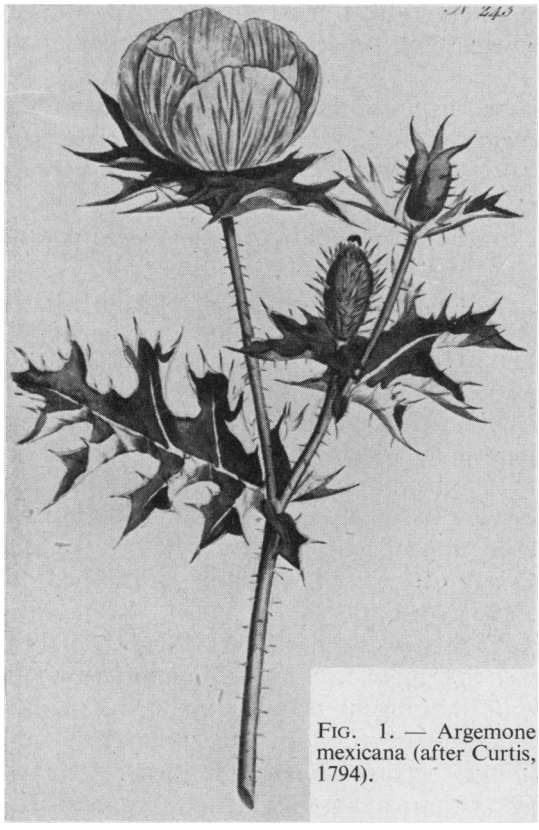

FIG. 1. — Argemone mexicana (after Curtis, 1794).

Argemone mexicana (or *Prickly yellow poppy*), being one of the most accommodating of weeds, has spread by zonal distribution and become naturalized during the last 350 years in all eastern tropical and sub-tropical countries, including Africa, India, South-East Asia, Australia and the Philippines, although it is rare along the American Pacific coasts (Prain, 1895).

The dispersal of this poisonous weed across half the globe reveals a fascinating story of man-induced followed by natural spread; but one which is also sad in that suffering and blindness has been caused to masses of the poor populations in the East and especially in India.

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The herb known as " Chicalotl " was used by the American Indians for its narcotic and other empirical virtues. John Gerard (1597), the English herbalist, first obtained its seeds in 1592, and successfully cultivated it as a garden plant in London. Bauhinus (1596), the taxonomist, correctly placed it among the papavers. Clusius (1601), falsely believing it to have Greek medicinal antecedents, described it among the rare plants of history. Morison (1680) described the herb, and his famous Herbarium at Oxford still has the original dry-plant specimen collected over 250 years ago. Tournefort (1694) first defined the herb's genus and species and named it *Argemone mexicana*. The species-name is misleading, for the plant originates from the Antilles and is only found in Mexico as an introduction at sea-ports.

The naming of the genus from the Greek word ἀργεμώνη has proved disastrous, for the name was associated in Greek classical medicine (Hippocrates, 5th cent. B.C.) with a " white speck " in the eye, and with certain poppy-like herbs with orange latex which heal diseases of the eye (Dioscorides, 1st cent. A.D.), whereas the American weed has been shown to cause disabling blindness.

The Portuguese, confounding the virtues of the Greek Argemone with the newly-introduced weed, and unaware of its potentially poisonous properties, introduced it into East Africa (Prain, 1895), and probably into India *via* the Portuguese settlement at Goa.

Besides botanical evidence, it can be shown that *Argemone mexicana* was foreign to India for the following reasons:

(*a*) it is absent from the Yunani Mughal Tibb system of Hakimi medicine prevalent in India in the 16th and 17th centuries (personal collection of manuscripts),

(*b*) it is not mentioned by Orta (1563), a scholarly medical writer very familiar with the drugs both of the West Indies and of Western India,

(*c*) it is not mentioned by Rheede van Drakenstein (1678) in his vast botanical work describing the very regions into which the weed later spread and established itself.

The existence of *Argemone mexicana* in India was first recorded by Burmannus (1768), the plant having been introduced some time before this date, probably in the first half of the 18th century. It has now established itself over the greater part of India and South-East Asia.

Ainslie (1813), who compiled the first (western) Materia Medica of India, recorded the Jamaican Argemone as a foreign or imported Dhatura (narcotic), and noted its use by practitioners of the indigenous Indian systems of medicine because of its alleged ophthalmic and other properties. Such use led to a second mis-identification of the herb with an unknown plant with yellow latex called *Svarnakshiri** (Charaka, B.C.) which was prescribed in the ancient Indian Ayurvedic system of medicine. Many modern Ayurvedic text-books perpetuate this mistaken identity; even since the discovery in India (Sarkar, 1926) that the seed-oil of *Argemone mexicana* produces epidemic dropsy and glaucoma, Ayurvedic practitioners continue to prescribe

*The real *Svarnakshiri*, like the real *Argemone*, because of its scarcity or extinction, remains unidentified.

this blinding poison, and recommend it as suitable for research (Hakim, 1950). The grave danger of this mistaken recommendation becomes evident when it is remembered that the Ayurvedic practitioners, both learned and ignorant, serve the medical needs of most of the vast village populations of the Indian sub-continent.

The historical study of this herb brings out the dangers of false plant identifications. Textual, historical, geographical, and morphological accuracy is essential for any real research into the empirical wealth of Ayurvedic drugs.

Epidemiology

Widespread epidemics of dropsy, at time affecting 7,000 persons and sometimes killing 1,500, have been frequently recorded in India since 1866. McLeod (1877) named the condition "epidemic dropsy", but its real cause remained unknown for another 50 years. The most constant signs were lower-extremity oedema, gastro-intestinal disturbances, and skin changes. Since 1909, an associated primary high-tension glaucoma, often leaving permanent defects in the visual fields, has been recorded. Various theories of malnutrition, infection, or food-intoxication were suggested, as the epidemics were localized and usually confined to poorly-fed populations. Sarkar (1926) was informed by villagers that a local epidemic was due to contamination of a cooking-oil press with seeds of *Argemone mexicana*. Kamath (1928) recorded a similar contamination of sesame oil used for cooking. These observations revealed the real aetiology of epidemic dropsy, linking the epidemiological records with the migration of *Argemone mexicana* into India.

Research workers in India have collected valuable material on the clinical, ophthalmological, chemical, biochemical, histological, and experimental aspects of the problem. Oedema and haziness has been produced in human volunteers, but not in animal experiments.

Epidemics have also been recorded in other tropical countries (Meaker, 1950), and argemone-poisoning has been noted in cattle (Hurst, 1942) and fowls (Hart, 1941; Steyn, 1950).

Chemistry

Argemone mexicana L. and its Seed-Oil.—The plant is known to contain several alkaloids including berberine and protopine in the herb (Santos, 1932), sanguinarine and dihydrosanguinarine in the seed-oil (Sarkar, 1948), and some undefined poisonous principles in the latex (Henry, 1949) and probably in other structures.

The present experiments have shown that increased ocular tension and pathological damage to the eyes of experimental animals was produced not only by argemone oil and its active alkaloid sanguinarine, but also by seed-oils, alkaloids, and capsule extracts from other papaveraceae.

A number of inter-related and inter-convertible alkaloids, all derivable from *iso*-quinoline, are found in the natural order *Rhoeadales*, which includes the papavers (Henry, 1949). These may explain the experimental activity of other papaver substances, and deserve fuller investigation, especially if idiopathic primary glaucoma is shown to be caused by indirect ingestion of papaver herbs through animal vectors.

Several physical and chemical tests for detecting argemone oil were devised by Lal and others (1939), Sarkar (1941), and Sen (1946). They are more valuable as negative evidence.

Sarkar (1948) isolated the two main alkaloids of argemone seed-oil, identifiable with sanguinarine and dihydrosanguinarine. Sanguinarine (0·44 mg./ml.) accounted for 5 per cent., and dihydrosanguinarine for about 87 per cent. of the total alkaloids present. Dihydrosanguinarine could be chemically oxidized to sanguinarine hydrochloride.

Bailey (1951) recovered 0·28 mg./ml. sanguinarine chloride m.p. 275–278° C. (decomp), and 4·6 mg./ml. dihydrosanguinarine m.p. 192-193°C. (decomp) from argemone oil.

Sanguinarine.—(ψ-Chelerythrine), C_{20} H_{13} O_4 N. H_2 O, is an alkaloid of the benzphenanthridine sub-group of the *iso*-quinoline group, naturally occurring in several of the *Rhoeadales* (Henry, 1949; Wehmer, 1929). Though known since 1829, the alkaloid has only recently become available, free from other substances. The natural alkaloid crystallizes in colourless needles, is insoluble in water, but dissolves in organic solvents. Sanguinarine gives a blue colour-reaction with concentrated H_2SO_4, and shows a blue-violet fluorescence, and its quaternary salts form water-soluble red crystals (Henry, 1949; Crawford, 1951).

Bailey, Robinson, and Staunton (1950) have studied its molecular structure and absorption spectra. The free-base sanguinarine, m.p. 268–270° C. (decomp), being insoluble, I have used throughout the present experiments the blood-red, long, thin, water-soluble crystals of sanguinarine chloride (C_{20} H_{13} O_4 N. HCl. 3 H_2 O), 7: 8: 2′: 3′-dimethylenedioxy-1: 2-benzphenanthridine methochloride. These were prepared by Dr. Bailey with slight modifications of the method of Sarkar (1948).

Toxicology

L.D. 50.—The dose of both argemone oil and sanguinarine lethal to 50 per cent. of animals was determined on groups of sixty mice after intravenous, subcutaneous, intraperitoneal, and oral administration. The results were recorded over a 48-hr mortality.

1 mg. BAL (2: 3-dimercaptopropanol), given intramuscularly 30 minutes before sanguinarine, the mice being kept at 17° C., seemed to protect the animals by raising the L.D. 50.

The L.D. 50 of both argemone oil and sanguinarine showed wide differences in a series of tests. This was due to a prolonged lethal action of the drugs, and consistent figures were obtained by assessing the L.D. 50 in 160 mice over a period of 15 days after a single injection of four different doses of each drug. The L.D. 50 of argemone oil was 0·9 ml./100 g., and that of sanguinarine 1·8 mg./100 g. intraperitoneally in mice. Lowered temperature (4° C.), did not influence the mortality in mice or tadpoles.

Relative Toxicity of Argemone Oil and Sanguinarine.—Comparison of the biological figures of L.D. 50 of argemone oil and sanguinarine with the chemical estimates of sanguinarine in argemone oil, showed that the oil was 4½ to 7 times more toxic, biologically, than could be chemically accounted for by the sanguinarine present.

Attempts to impregnate bland oils artificially with chemical equivalents of sanguinarine, for biological comparison, were unsuccessful, as sanguinarine was rendered inert. Attempts to show that dihydrosanguinarine could account for

the greater toxicity of the oil were also unsuccessful, as large doses of dihydrosanguinarine (40 mg./100 g.), injected as a suspension in arachis oil, were non-toxic.

Dihydrosanguinarine, although chemically convertible to sanguinarine, and seventeen times more abundant in argemone oil, seems to be either inert or rendered insoluble and inert by chemical extraction. If intrinsically inert, there must be some other toxic factor in argemone oil, besides dihydrosanguinarine and sanguinarine.

Apart from the natural variations in samples of argemone oil and the recognized diminution of its toxicity by heat, light, and ageing, the above factors of delayed lethal action, changes in the solubility of its active principles, and the natural resistance of the host, complicate the picture. Some of these may explain the conflicting figures (Sarkar, 1941) of the toxicity of argemone oil in human and animal experiments given by previous workers (Lal and Roy, 1937; Lal and others 1939, 1941; Pasricha and others, 1939, 1940; Chopra, 1939).

Single Lethal Dose of Sanguinarine.—3 to 4 mg./100 g. sanguinarine, injected subcutaneously, was lethal to adult rats, and 10 mg./kg. to rabbits.

Sub-acute Sanguinarine Toxicity.—2·5 mg./100 g. sanguinarine, injected daily subcutaneously, produced diarrhoea and enteritis, in mice, and killed them in 3 to 8 days. 1 mg./100 g., injected daily intraperitoneally, produced some signs of ascites and enteritis in rats and killed them in 7 to 15 days.

Chronic Toxicity in Young Rats.—A group of ten young rats was kept on an oral daily dose of 1 ml./100 g. argemone oil mixed into the rat-cubes; another ten rats received 0·35 mg./100 g. sanguinarine daily, freshly dissolved and mixed in food. Ten were kept as controls. Five rats in both poisoned groups received in addition 1 g. dried yeast per rat daily. All rats survived the treatment for 225 days, although the growth of the poisoned rats was arrested (c.f. Sarkar, 1941).

Chronic Toxicity in Old Rats.—Three groups of old rats were maintained in the same manner and with the same doses for 250 days. Yeast was omitted. Towards the termination, a third of the rats in all three groups died, probably from senility rather than lethal toxic action. The poisoned animals of this series showed marked retinal damage.

Both the young and the old poisoned rats showed apathy, motley fur, and keratodermatitis. The dermatitis did not show ultra-violet fluorescence like the lesions of pantothenic acid deficiency. Yeast partially prevented the dermatitis. Relevant findings from both these experiments are incorporated in other sections.

Pharmacology

Meyer (1892) investigated some of the properties of sanguinarine, but not in association with the argemone oil problem. Most of his findings have been corroborated and many additional pharmacological properties of sanguinarine discovered in the present study.

Sanguinarine depresses the actions of sympathetic stimulation and of adrenaline.

(*a*) The left splanchnic nerve of cats under chloralose anaesthesia was exposed and electrically stimulated by condenser discharges at intervals of 5 minutes. Each stimulation produced a fairly constant transient rise in the blood pressure, averaging 75 mm. Hg. Sanguinarine was injected intravenously, and the splanchnic re-

stimulated 1 minute after the injection. 1 mg. sanguinarine depressed the effect of subsequent stimulation to 65 mm., 2 mg. to 54 mm., and 3 mg. to 14 mm. blood pressure. Although proportional to the dose at first, the depression later became complete and irreversible. The action suggested a progressive blocking of the constrictor receptors by sanguinarine.

(*b*) Isolated rabbit ears were perfused through the central artery with oxygenated Locke's solution, and the venous outflow collected and measured by a volume recorder unit. Addition of 0·04 μg. adrenaline into the perfusate caused a temporary constriction of the vessels as recorded by a decrease in outflow. Addition of sanguinarine in doses up to 0·1 mg., had no direct effect on the rate of outflow. When adrenaline was added after a dose of sanguinarine, its constrictor effect was greatly diminished.

(*c*) A series of constant depressions, produced by doses of 2 μg. adrenaline on the rabbit's isolated duodenum were recorded. Addition of 0·2 mg. sanguinarine produced no direct depressant or stimulant action on the duodenum. When doses of adrenaline were added after a dose of sanguinarine, their effect was progressively and irreversibly diminished.

Higher concentrations of sanguinarine directly depressed the rhythmic movements of the gut, which were restored neither by washing out nor by additions of pilocarpine or acetylcholine.

Sanguinarine first stimulates and later depresses adrenaline-like actions.

(*a*) A series of intravenous injections of sanguinarine, from 1 to 4 mg., into a chloralosed cat, at first produced small rises in the blood pressure. Later, the identical range of doses injected into the same animal produced relatively larger falls in blood pressure.

The reversal of the action of sanguinarine on the blood pressure, may be due to primary stimulation of adrenaline constrictor receptors, which are prominent, followed by their blockage and subsequent action on the dilators, thus resulting in fall of blood pressure.

(*b*) Small doses of sanguinarine added to isolated rabbits' hearts, rabbits' auricles, and frogs' hearts, at first caused a slight increase in amplitude, frequency, and coronary outflow. Subsequent additions of sanguinarine progressively decreased amplitude. frequency, and coronary flow.

Action of sanguinarine abolished or reduced by adrenaline.

(*a*) Intravenous injections of 0·05 to 1 mg. sanguinarine in anaesthetized guinea-pigs, produced rapid broncho-constriction proportional to the dose, as recorded by the method of Konzett (1940). Five μg. adrenaline, injected intravenously 45 seconds before the injection of 1 mg. sanguinarine, abolished its broncho-constrictor effect. 0·5 μg. *iso*-prenaline, or 1 mg. anthisan, or 10 μg. atropine similarly administered, reduced the subsequent effect of sanguinarine.

(*b*) The ocular tension-raising property of local sanguinarine or argemone oil was greatly diminished by the action of adrenaline.

Sanguinarine decreases the action of acetylcholine.

(*a*) A series of applications of acetylcholine in a concentration of $3 \cdot 3 \times 10^{-7}$, produced a constant temporary depression in amplitude and rate of isolated rabbit auricles. When sanguinarine $6 \cdot 6 \times 10^{-6}$ acted on the auricles, the depressant

effects of subsequent applications of acetylcholine were progressively reduced. Repeated applications of sanguinarine, or higher concentrations, produced both progressive and irreversible decreases in the amplitude and rate, and cessation of contraction. Prolonged application of sanguinarine to isolated rabbit and frog hearts produced both progressive depression of amplitude and rate, and insensitivity to acetylcholine.

(*b*) A series of equal contractions of the virgin rat uterus were obtained by applications of 0·8 mg./litre carbachol at regular 2-minute intervals through an automatic relay system. When sanguinarine $2 \cdot 5 \times 10^{-5}$ acted on the uterus and was washed out, the contractile effect of subsequent additions of carbachol was progressively reduced.

(*c*) Normal contraction of the frog rectus muscle produced by acetylcholine 2×10^{-3} was progressively diminished by the prior addition of sanguinarine 5×10^{-6}. Sanguinarine produced a partial contraction, followed by imperfect relaxation, leading to permanent contracture and acetylcholine insensibility.

(*d*) The electrically stimulated frog sciatic-gastrocnemius probably contracts by release of natural acetylcholine. When sanguinarine 10^{-4} acted on the muscle, it progressively reduced the subsequent electrical contractions and led to irreversible contracture.

Some of the effects sanguinarine suggested an inhibition of cholinesterase, whereby spontaneously generated acetylcholine remained undestroyed, producing a prolonged acetylcholine effect.

Other Effects.

(*a*) Injections of sanguinarine produced a miotic effect on the pupils of mice, as determined by the method of Grewal (1951). The miotic effect was determined by counteracting the mydriatic effect of a constant dose of atropine previously given to each animal. The miotic effect of sanguinarine on mice (and rabbits) may be explained by either an antagonism of local adrenaline, or an inhibition of cholinesterase.

(*b*) The anaesthetic activity of sanguinarine was demonstrated by the production of plexus anaesthesia in frogs, and intra-dermal anaesthesia in guinea-pigs (Method of Bülbring and Wadja, 1945). A concentration of 0·05 per cent. on the frog plexus produced anaesthesia in 5 minutes followed by rapid and irreversible paralysis.

(*c*) Sanguinarine did not influence the rate of effective stimulation of isolated, electrically-driven rabbit auricles (Method of Dawes, 1946).

Similarities between sanguinarine and the cinchona alkaloids (especially quinidine):

(1) they depress the action of adrenaline on rabbit ear vessels, and cat blood pressure and hyperglycaemia;

(2) they depress the actions of acetylcholine on rabbit auricles, rabbit duodenum, rat uterus, and frog rectus;

(3) they produce local anaesthesia and destructive lesions in nerves and retinae.

Action of sanguinarine and the theory of local hormones.—The demonstration of spontaneous synthesis of adrenaline and acetylcholine in several isolated tissues, and of the delicate mechanism by which such synthesis is adequately balanced by local enzymes which destroy them, has led to the local hormone theory. This also

explains the apparently contradictory or reversible actions of adrenaline and acetylcholine (Burn, 1950 a, b.). The actions of other extraneous drugs like sanguinarine can be explained by substrate competition with the local hormones at various stages of their synthesis, action or destruction.

Biochemistry

Sanguinarine enhances the action of insulin.

The blood glucose after injection of 1 unit insulin into a series of rabbits, was estimated at hourly intervals for 5 hours. Each reading was expressed as a percentage of the initial blood glucose, and the mean of these determined for five animals. A mean blood glucose reduction of 40 per cent. of the initial value was observed. Two days later, 5 mg. sanguinarine was injected subcutaneously into the same group of animals 30 minutes before the injection of 1 unit insulin. The reduction of mean blood sugar increased to 53 per cent. No convulsions occurred when the rabbits received insulin alone. All were convulsed and showed venous collapse when they were given both sanguinarine and insulin. It has been observed that sanguinarine alone failed to produce any convulsions in rabbits.

The recovery of the blood glucose after a hypoglycaemia caused by insulin is known to depend in part upon the release of adrenaline from the adrenal glands, which causes a discharge of glucose from the liver. The increase in the action of insulin due to sanguinarine might therefore be explained by a diminished effectiveness of adrenaline.

Sanguinarine and blood pyruvate.

An increase in blood pyruvate in patients with epidemic dropsy resulting from argemone oil was noted by Wilson and Ghosh (1937). Sarkar (1948) showed that sanguinarine was toxic to several enzyme systems, especially to pyruvate oxidase, and produced a rise in blood pyruvate level in acutely poisoned rats. Pyruvate accumulates in blood and tissue fluids in vitamin B1 deficiency (Peters, 1936). Thompson (1952) suggested that pyruvate oxidase blockage by sanguinarine might explain the dropsy seen in argemone oil epidemics, which was formerly thought to be a type of beri beri.

The pyruvate blood level of all the rats poisoned with either argemone oil or sanguinarine during 225 days (see " Toxicology ") was determined immediately after they had been killed. There was no consistent rise in pyruvate in the chronically poisoned groups compared with the controls, nor any modification in the groups receiving poison plus yeast. Blood pyruvate may only be raised in acute poisoning with very high, almost lethal, doses of sanguinarine.

Sanguinarine on acetylcholine synthesis and breakdown.

Several pharmacological effects of sanguinarine could be explained by its action on either the synthesis or breakdown of tissue acetylcholine. A biochemical approach to this problem was attempted by the following experiments:

(*a*) A homogenized extract was made from rat cerebral hemispheres normally known to contain cholinesterase. To this extract sanguinarine was added *in vitro*. Another litter-mate rat was injected subcutaneously with sanguinarine and killed after an hour, and a similar brain extract was prepared. The extracts were separately incubated in Warburg respirometer flasks, with proper controls, acetyl-β-methyl choline chloride being used as substrate. The CO_2 evolved was measured and the course of the reaction graphed. Sanguinarine *in vitro* (0·33 mg./ml.) interfered with the anaerobic production

of acetic acid in the brain tissue, and inhibited the enzymic hydrolysis of the substrate by 60 per cent. in 30 minutes. Sanguinarine (5 mg./200 g. rat) injected *in vivo*, had no effect on the cholinesterase activity of brain extract.

(*b*) In another quantitative experiment, sanguinarine *in vitro* in a concentration of 0·33 mg./ml., inhibited the same reaction by 62 per cent., and a tenth of that concentration caused 25 per cent. inhibition.

(*c*) Sanguinarine 0·33 mg./ml. inhibited the enzymic hydrolysis of benzoyl choline by natural pseudocholinesterase in horse serum by 64 per cent. in 30 minutes. A tenth of that concentration caused a 40 per cent. inhibition.

(*d*) In view of the inhibiting action of sanguinarine on choline acetylase and pyruvate oxidase, both of which require the presence of Co-enzyme A, and the protection by BAL against sanguinarine toxicity (probably by protecting the –SH group of Co-enzyme A from oxidation), an investigation was made on the effect of sanguinarine on acetylation. For this purpose, the acetylation of sulphanilamide was studied, in both the presence and the absence of sanguinarine.

Two groups of four rats were kept in metabolism cages and their urine collected. All the animals received sulphanilamide in a dose of 10 mg./100 g. per 24 hrs in their food for 5 days. One of the groups was also injected subcutaneously with 1 mg./100 g. sanguinarine. The pooled urine from both groups was tested daily for its total free and acetylated sulphanilamide contents. No consistent difference was detected between the two groups. Sanguinarine, in the dose used, did not affect the acetylation of sulphanilamide.

Sanguinarine inhibits adrenaline catabolism.

Several of the pharmacological, biochemical, and ophthalmological effects of sanguinarine could be explained by its interference with the actions of adrenaline. Sanguinarine, as an alkaloid with the *iso*-quinoline nucleus, is considered as a possible product of the amine-oxidase reaction (Blaschko, 1952), and may have a high affinity for amine-oxidase.

The problem was investigated by a study of amine-oxidase activity by the Warburg manometric technique. The amine-oxidase was prepared from guinea-pig liver, a source of the active enzyme, and L *p*-Sympatol was used as substrate.

Sanguinarine 0·5 mg./ml. inhibited the enzymic oxidation by 57 per cent. in 45 minutes. A tenth of this concentration produced no inhibition. It is unlikely that such high concentrations of sanguinarine could be found in human body fluids in epidemic dropsy.

Production of ascites and skin oedema by sanguinarine.

Epidemic dropsy is characterized by oedema confined to the dependent limbs (rarely generalized) and glaucoma. Chopra and others (1935) found a lowering of the plasma proteins in epidemic dropsy patients with lowered serum albumin, but raised serum globulin levels. Pasricha and others (1938) found a lowering of the specific gravity of the serum in epidemic dropsy patients. The serum specific gravity was observed to return to normal with the disappearance of the oedema. Oral feeding with cooking oils suspected of contamination with argemone produced oedema in human volunteers after 9 to 23 days (Lal and Roy, 1937), and Chopra (1936) similarly produced marked oedema with pure argemone oil. Oedema of the wattles of fowls has been induced by feeding with argemone seeds (Hart, 1941; Steyn, 1950).

During the present study I have shown that sanguinarine can rapidly produce ascites and skin oedema, in 7 to 10 days in rats kept on a low (0·7 per cent.) protein diet consisting exclusively of carrots and water *ad lib.* This was a modification of

the hypo-proteinaemia oedema and ascites (Dicker and others, 1946) produced in 60 per cent. of rats after about 36 days on a diet of 85 per cent. carrots plus a mixture of salts, starch, fats, and vitamins.

Four groups of ten rats were kept on the exclusive carrot diet. One group received sanguinarine 1 mg./100 g. subcutaneously each day in the skin of the dorsum. Another group received the same dose of sanguinarine plus an extra independent injection of 2·5 mg./100 g. BAL. The third group was injected with argemone oil 0·5 ml./100 g. subcutaneously daily. The fourth group was kept as a control. The rats were killed on the 9th day after seven injections. No ascites was seen in the control group. Ascites was very marked in all rats injected with sanguinarine, absent in those injected with sanguinarine plus BAL, and very slight, but definite, in those receiving argemone oil, which may not have been fully absorbed, as evidenced by the formation of subcutaneous nodules.

Approximately equal discs of abdominal skin from each animal were punched out with a large cork borer, pooled for each group, weighed moist, and re-weighed after complete evaporation of fluid in a drying oven. The moisture content of the skin discs relative to their dry weight showed an increase of 145 per cent. in the controls, 600 per cent. with sanguinarine, 233 per cent. with sanguinarine plus BAL, and 214 per cent. with argemone oil. BAL therefore had a definite protective action.

In another experiment with paired feeding and the same diet, one group was injected with sanguinarine, another received sanguinarine plus choline chloride 200 mg./rat daily, and the third was kept as a control. Slight ascites was noted in both poisoned groups. The percentage increase over the dry skin weights was 156 per cent. in controls, 246 per cent. with sanguinarine, and 222 per cent. with sanguinarine plus choline. The group receiving choline consumed less food than the others. This dose of choline had no protective action, but appeared to be toxic perhaps through the reduced food intake.

The following conclusions have been reached:

(*a*) Sanguinarine interferes with the actions and catabolism of adrenaline and acetylcholine. BAL partially antagonizes sanguinarine.

(*b*) Animals can withstand enormous total doses of argemone oil or sanguinarine distributed over long periods. This presumes a detoxification mechanism, capable of continual functioning below an overwhelming threshold. The mechanism is probably connected with diet and blood protein. It may also explain the effects on specific tissues, such as the retina, and the acute rises in ocular tension which were seen only when the drugs were injected so close to the eye that they were not rapidly detoxified (*see* " Ophthalmology ").

(*c*) The protective protein factor increases the animal's resistance to the onset of ascites and oedema resulting from the toxicity of sanguinarine and argemone oil. In natural epidemics argemone oedema shows a predilection for populations poorly-fed on low (rice) protein.

(*d*) It is possible that the amino acid tryptophane is responsible for such protection, as it is known to be essential for plasma-protein formation. Tryptophane deficiency is known to produce changes in epithelia, cardiac muscle, and large liver-cell nuclei, comparable with argemone or sanguinarine pathology. Again,

close structural similarities with sanguinarine may explain how the latter displaces tryptophane by substrate competition and produces a picture of tryptophane deficiency.

(*e*) The technique of accelerating the onset of oedema in animals kept on a low protein diet by certain toxic substances such as sanguinarine, appears to be a valuable new method of investigating the activity of known or suspected oedema-producing substances, and may also prove a useful biological test for detecting unknown plant substances suspected of raising ocular tension.

Histo-Pathology

Previous work on the histo-pathology of epidemic dropsy stressed the marked dilation of the capillaries in the skin, pericardium, peritoneum, and iris, without leucocytic infiltration (Shanks and De, 1931; Chopra and others, 1935; Kirwan, 1935). Toxic doses in guinea-pigs and mice produced acute haemorrhagic glomerulo-tubular nephritis with vascular changes and congestion, thrombosis, and fatty liver degeneration (Pasricha and others, 1940). Acute cases showed extravasation of blood (Lal and others, 1941; Sarkar 1948).

In the present study, a large number of organs of animals poisoned with acute, sub-acute, or chronic doses of argemone oil or sanguinarine were studied.

Groups of several rats were injected with sanguinarine as follows:

2–3·5 mg./100 g. on one day;	0·5 mg./100 g. daily for from 7 to 13 days;
2·5 mg./100 g. for 2 days;	1 mg./100 g. daily for 15 days.

Acutely poisoned rats, despite almost lethal doses, showed surprisingly slight histopathological changes which could account for the high toxicity of either drug. Damage to the kidneys and adrenals was common but not extensive. The skin showed slight oedema. Eye damage was limited to degenerative corneal changes in Descemet's endothelium and the corneal epithelium.

Poisoned rats on low-protein diet showed an accentuation of the pathological changes. The abdominal skin was markedly oedematous below the muscle layer. Early degenerative changes were seen in the retina and cornea. Marked changes were observed in the liver, where the cells, nuclei, and nucleoli were large, and the nuclei contained more nucleoli than in the controls. The basic protein of the cytoplasm was reduced in amount, and the ribonucleoprotein of the nucleoli and cytoplasm was unaffected. The liver changes suggest toxic inhibition of plasma-protein formation.

Chronically poisoned young rats showed no consistent changes in the vascularity, blood vessels, elastic tissue, or epithelium of the skin. The adrenal zona glomerulosa showed dilated blood vessels; the medullary cells, with large heavily-stained nucleoli, suggested the possibility of hyperactivity, thus indicating effects on adrenaline formation within the nucleolus. In the eye, Descemet's endothelium showed either swelling or shrinkage posterior to the nucleus, or pyknotic changes in the nuclei. The corneal epithelium showed an increase in numbers of superficial stratified cells with deeply stained crenated nuclei and numerous mitotic figures. The fine elastic fibres, forming the prolongation of the Descemet's membrane towards the ciliary body, had disappeared in the poisoned rats.

Chronically poisoned old rats showed marked degenerative changes in the peripheral and central portion of the retinae. The degeneration, when present, was either complete, peripheral, central, limited to one side of the retina (Fig. 2, overleaf) or to one eye only. The pigment cells were detached or absent in places, and elongated, with swollen, highly vacuolated or pyknotic nuclei. The rod nuclei were pyknotic and showed destruction of outer and inner segments. The rods were intermingled with the bipolar and ganglion cells. The

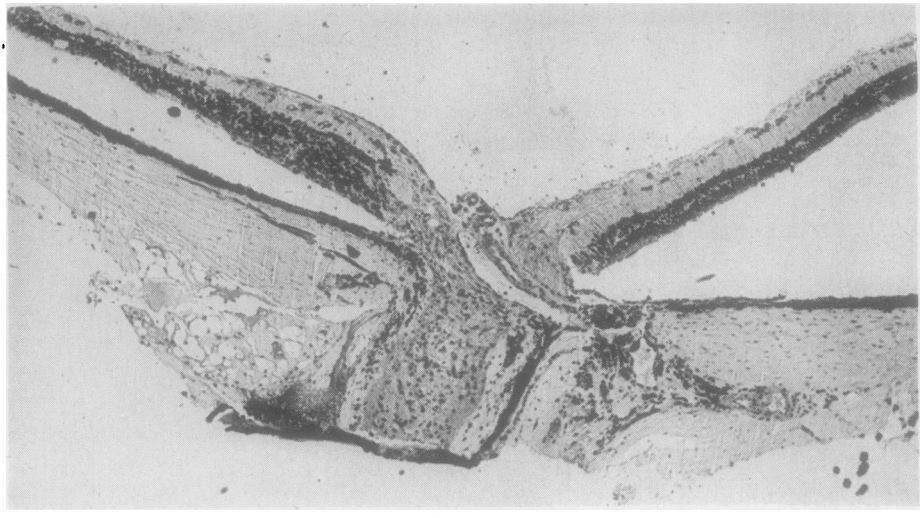

FIG. 2.—Degeneration of retina on left side by chronic oral argemone-oil poisoning in old rats.

bipolar and ganglion cells showed abnormal nuclei, with one or more pale basophil granules nearly filling the whole nucleus, and sometimes simultaneously with a large acidophil nucleolus. Similar nuclear changes were seen in pigment cells and in neuroglia cells of the optic nerve. Descemet's endothelium and the corneal epithelium showed degenerative changes. The retinal changes were more intense in rats who received 1 ml./100 g. argemone oil orally for 250 days, than in those who received an approximately chemically equivalent dose of sanguinarine 0·35 mg./100 g. No such changes were seen in the eyes of the old rats of the control group. The kidneys showed eosinophilic and hyaline casts in the cortex, loop of Henle, and collecting tubules. Fibrosis, lymphoid infiltration, and possibly hypertrophy of the juxta-glomerular apparatus, were noted. Degenerative changes and fibrosis of the blood vessels were seen in the testes.

The disproportion between the lethal action and the apparent lack of detectable morphological change, especially in acute toxicity, suggest a biochemical lesion which becomes lethal before morphological changes are manifest. The lack of gross morphological changes, even after prolonged poisoning, suggests a detoxification mechanism, possibly dependent on a normal protein metabolism, as skin and other changes were rapidly precipitated on a low-protein diet. The eyes, especially of senile rats, seem to be particularly vulnerable, and show similarities to quinine poisoning.

Ophthalmology

Previous Records.—Maynard (1909) first noted increased ocular tension in 100 patients with epidemic dropsy in India. Kirwan (1935) detected a primary, non-inflammatory, bilateral, high-tension glaucoma in 10–12 per cent. of epidemic dropsy cases, aged 20–35 years. The condition was also found in patients with slight or no signs of epidemic dropsy. He noted infrequency of pain, dim vision, slight haloes, and corneal œdema, without inflammation of the conjunctiva and sclera. The ocular tension was greatly raised and declined after some weeks. Cupping and optic nerve atrophy were a late phenomenon, due to tension, and preventable by early surgical intervention. Increased albumin and a toxic histamine-like substance were found in the

aqueous humour. Histological studies showed absence of cellular or fibrous changes at the filteration angle or drainage channels, but an intense, non-inflammatory capillary dilatation of the whole uveal tract, followed by increased endothelial permeability. The toxin showed a high predilection for both intra-ocular and body endothelium.

Other workers (Mukerjee, 1929; Chopra and others, 1935; Pasricha and others, 1939; Sanyal, 1942, 1951) recorded similar findings and noted retinal hæmorrhages, with low tension from the beginning or recurrent peaks of high tension.

Dimness of vision was noted by Meaker (1950) in argemone seed poisoning in Mauritius, and was also produced by experimental feeding with argemone oil in human volunteers (Chopra and others, 1939).

Action of Sanguinarine on the Drainage Mechanism of the Isolated Ox-Eye in a Perfusion Apparatus.—To study the direct action of drugs on the drainage mechanism of the anterior chamber of the isolated eye, independent of nervous and circulatory influences, I devised a perspex perfusion apparatus (Fig. 3).

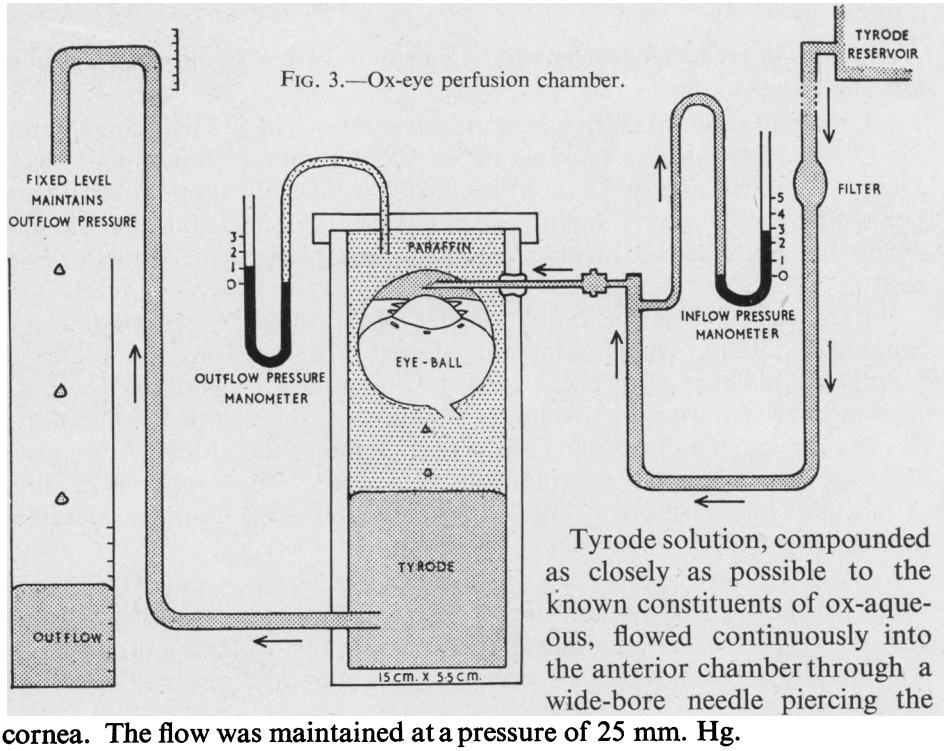

FIG. 3.—Ox-eye perfusion chamber.

Tyrode solution, compounded as closely as possible to the known constituents of ox-aqueous, flowed continuously into the anterior chamber through a wide-bore needle piercing the cornea. The flow was maintained at a pressure of 25 mm. Hg.

The eye-ball was suspended in the top half of the apparatus, surrounded by a bath of liquid paraffin, maintained at a pressure of 10 mm. Hg to simulate normal venous back-pressure. The tyrode solution entering the eye flowed out *via* the drainage channels, through the paraffin, and out of the apparatus.

After reaching an equilibrium, the outflow from the apparatus was recorded.

The mean outflow from fresh ox-eyes was 1 to 4 ml./hr. This was close to the known physiological rate. The flow was less with eyes of old animals, rabbits, monkeys, and man.

Direct injections of drugs or dyes, with a long thin needle running through the wide-bore needle, could be made into the anterior chamber of the eye without disturbing the balance of the whole system. Dyes emerged 4 minutes after injection at four cardinal points on the surface of the eye just behind the cornea-scleral junction, probably from the openings of the episcleral veins. Some drainage was also seen behind the equator.

Injections of 0·1 to 2μg. adrenaline, nor-adrenaline, or acetylcholine did not influence the outflow. Sanguinarine 20 to 100 μg. diminished the outflow from 15 to 40 per cent. The decrease occurred during the first 15 minutes after injection, and the outflow slowly returned to normal. The decrease over 15 minutes was comparable to the peak rise in ocular tension produced by injections into the eyes of living rabbits. This suggested that sanguinarine exerted a pharmacological constrictive influence on the outflow channels.

Attempts to produce Glaucoma-like Changes in Live Animals with Argemone Oil and Sanguinarine.

(*a*) A rabbit received daily subcutaneous injections of 2·5 mg. sanguinarine for 15 days. Another received a total of 100 mg. during 60 days in doses of 5 mg. The ocular tension was measured by a Maclean's tonometer several hours after each injection, but no rise in tension was detected in either animal during the entire period. Only local anaesthesia (1 per cent. anethaine) was used.

(*b*) The basic ocular tension in a young rabbit was measured by a Schiötz tonometer. 10 mg. sanguinarine was injected subcutaneously and a series of tonometric readings were taken every five minutes for 2 hours. The basic tension of 17 mm. rose to 32 mm. in 30 minutes and returned to normal in 2 hours. The animal died after 12 hours. In another rabbit, two 5-mg. doses of sanguinarine injected subcutaneously produced 5-mm. rises in tension. A subcutaneous injection of 2 mg. sanguinarine into a guinea-pig produced an immediate small rise in tension.

(*c*) A rabbit was wrapped up, and one eye was anaesthetized by Anethaine drops and kept under a tonometer. 8 mg. sanguinarine was injected into the ear vein. There was a rapid rise in the tension which reached a maximum of 13 mm. above the basic level within a few minutes and returned to normal in 15 minutes.

(*d*) Two groups of ten young rats were each given, mixed in their food, either 1 ml. argemone oil or 0·35 mg. sanguinarine daily for 225 days (*see* " Toxicology "). Half of the animals in each group also received 1 g. dried yeast. Ten rats were maintained as controls. The ocular tension was measured in each animal with a specially lightened Maclean's tonometer after

local anaesthesia with anethaine. A serial record of tension, measured during the course of the experiment, showed an apparently slight increase of tension in the poisoned animals.

A series of ophthalmological and retinoscopical examinations was carried out on each animal on several occasions during the experiment. The pupil was dilated by drops of homatropine plus cocaine 2 per cent., instilled 30 minutes before each examination. The optic disc, with the site and degree of any pallor, greying, edge or suspicious or definite cupping, was observed and recorded for each eye.

Mr. Lloyd and Dr. Boyd alternately examined the eyes, without pre-knowledge of each rat's group. Their observations were usually consistent with their own previous findings and with each other. Among the twenty poisoned rats, progressive cupping was noted in thirteen eyes and suspicious changes in ten more. Some control eyes showed a " physiological " cup.

A series of retinoscopic examinations made on these animals showed a tendency towards increasing hypermetropia or hypermetropic astigmatism in the horizontal axis. This is a type of change similar to that in human buphthalmos in which increasing intra-ocular pressure distends the globe with reduction of the radius of curvature of the cornea.

As the eyes of some poisoned rats seemed to be more protruding than those of the controls, the rats were killed at the end of the experiment, and each eye-ball was dissected and weighed. A comparison of the total eye weights of each group showed an increase in weight among the poisoned rats, which was possibly just statistically " significant ". Yeast had no protective effect in any of the tests.

The eyes were fixed in Bouin's fluid, each lens was carefully removed, and serial sections were cut at 5μ and examined for histo-pathological changes. The section through the middle of the optic disc was determined by selecting that with maximum distance between the outer nuclear layer of the retina, intercepted by the optic nerve. Microscopic examination showed no conclusive evidence of cupping of the type seen in human sections, nor was there any consistent relationship between the depth or shape of the optic nerve depression and the previous clinical findings. The blood vessels entering through the optic nerve often distorted the picture.

The rat eye lacks a lamina cribrosa. The clinical appearance of cupping may have been due to glial proliferation. Unfortunately no histological technique is available for demonstrating this on a quantitative basis.

(*e*) The previous experiment on young poisoned rats had indicated a tendency towards raised tension, clinical evidence of cupping, hypermetropic astigmatism, and increased weight of the eye-balls (due to swelling). This suggested another chronic experiment on old rats, in which, the eyes being less yielding, any rise in tension would precipitate more conclusive clinical and histological evidence of glaucoma-like changes. Two groups of old rats were similarly fed on either argemone oil or sanguinarine for 250 days, a third group being kept as controls (see "Toxicology"). Tonometry gave

inconsistent results. Serial ophthalmological examinations among the ten poisoned rats showed consistent and progressive cupping in six eyes and suspicious cupping in nine more. Retinoscopic examination, less consistent than in the previous experiment, showed similar hypermetropic astigmatism, but greater basic abnormality. The eye-ball weights showed no statistical increase among the poisoned animals.

A similar detailed histological study was made on serial sections of the eye-balls of each rat. No confirmation of the clinical evidence of cupping could be obtained.

A histological finding of great interest was detected in the retinae of several of the poisoned rats in this series of old animals. There was marked disorganization and degeneration of the nuclear layers of the retina, which was either complete, peripheral, central, or limited to one side only. The argemone-oil group showed maximum degeneration, and this was less evident in the sanguinarine and absent in the control group.

The retinal toxicity of argemone oil and sanguinarine in old rather than young rats, may have some bearing on the age-incidence problem of primary glaucoma, should it prove to be toxic in origin. This toxicity has pharmacological and clinical parallels with the action of quinine and quinidine.

Acute Rise in Ocular Tension produced by Subconjunctival Injections of Argemone Oil or Sanguinarine in Rabbits.—As a result of my observations that sanguinarine caused a diminution in the perfusion outflow when directly introduced into the anterior chamber of the isolated ox-eye, Mr. Lloyd suggested the injection of sanguinarine subconjunctivally in live rabbits, based on the known absorption of penicillin from under the conjunctiva.

Sanguinarine or argemone oil in small quantities (0·1–0·2 ml.), injected subconjunctivally into one eye of each of a series of rabbits, produced a remarkable rise in ocular tension. The other eye, which was injected with inert control substances, water, bland oils, etc., showed no such rise.

The rabbits required no anæsthesia, apart from a few drops of 1 per cent. anethaine (amethocaine hydrochloride), dropped into the eye before and several times during the examination. They were wrapped up in cloths and held with the cornea horizontal. A basic reading of the normal tension was taken with a Schiötz tonometer before each injection. After the injection a series of readings were taken at intervals of a few minutes (Fig. 4).

When active substances were injected, there was a rise in tension beginning from 3 to 10 minutes after injection and coming rapidly to a peak in 15 to 20 minutes. Some eyes maintained a high level of tension for 30 to 50 minutes, but usually there was a decline after 20 minutes and basic levels were reached in 40 to 90 minutes. After a single injection, the tension was well below the basic level on the following day and remained sub-normal. The tension did not drop much below the basic 24 hours after the injection of inactive substances. Active substances often produced marked conjunctival inflammation and some corneal haziness.

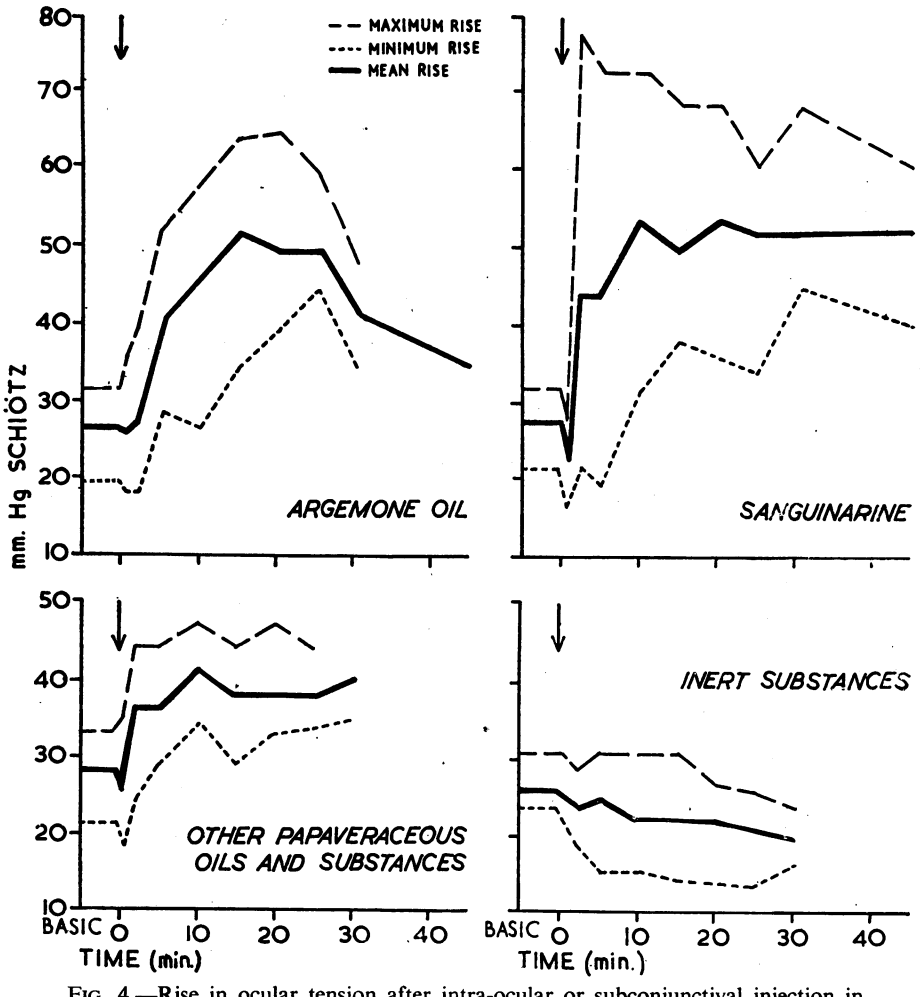

FIG. 4.—Rise in ocular tension after intra-ocular or subconjunctival injection in rabbits, showing average of ten typical records.

The basic tension in rabbits ranged from 20 to 35 mm. (average 28).

Argemone Oil (over 22 tests) raised the basic tension by an average of 25 mm. above the basic level (maximum 43 mm).

Sanguinarine (over 19 tests) 0·04–0·4 mg. in 0·2 ml. water raised the tension to an average 25 mm. above the basic level (maximum 38 mm.).

Inert Substances (olive, tea-seed, rape-seed, and gingelli oils, seed-oils from the opium poppy (*Papaver somniferum* L.) and certain other poppies, distilled water, priscol, etc.) produced insignificant or no change in the basic tension.

Active Substances that raised the basic tension were found in:

(*a*) capsule extract of *Papaver dubium* L.,

(*b*) seed-oils of certain papavers—*Eschscholtzia californica* Cham. (Californian poppy), *Papaver argemone* L., and *Papver rhoeas* L. (Cornfield poppy),

(*c*) the alkaloid chelerythrine.

Results of Subconjunctival Injection in Other Species.—Injections of sanguinarine 0·4 mg. into two Malayan Macaque monkeys, under generalized nembutal or urethane anæsthesia, produced rises of over 10 mm. above the basic level. Argemone oil 0·1 ml. also produced the same effect. The anæsthetics may have diminished the rise in tension.

Acute Rise in Ocular Tension produced by Anterior-Chamber Injections of Argemone Oil or Sanguinarine in Rabbits.—To avoid the severe inflammation and fibrosis of the ocular conjunctiva, the drugs were injected directly into the anterior chamber of the eye. The animals were prepared as for the subconjunctival technique. Minute quantities (0·01–0·02 ml.) of either the reactive or control substances were injected with a special syringe and a very fine needle through a long trans-corneal approach into the anterior chamber. An equal volume of aqueous was drawn back into the syringe to re-establish a constant pressure in the eye, and the needle was removed. Successive tension readings were taken immediately after, and at intervals of a few minutes after the injection (Fig. 4).

Argemone oil, sanguinarine, and other papaveraceous substances caused an acute rise in tension, and several control substances were found to be inert. The general slope of the graph was similar to that of the subconjunctival technique, except for an initial drop in the few cases in which aqueous escaped. The rise was usually quicker, and relatively small doses were effective. There was an immediate fall in tension in the few cases of experimental leaks from the needle track, which usually returned to base in 15 to 20 minutes. With inert substances the tension remained at this basic level whilst it continued to rise with active drugs. Very few animals were insensitive.

Argemone oil (46 tests) 0·01–0·02 ml. raised the basic tension by an average of 25 mm. (maximum 45 mm.).

Sanguinarine (20 tests) 4–400 μg raised the basic tension by an average of 25 mm. (maximum 55 mm.).

Inert Substances (20 tests; olive, tea-seed, and gingelli oil, water, 0·4 μg ethanol, 1–40 μg adrenaline in oil, etc.) produced no significant change in tension.

Active Substances (35 tests) that also raised the basic tension were found in:

(*a*) capsule extracts of *Argemone mexicana* L., *Papaver rhoeas* L., *Papaver dubium* L., *Chelidonium magus* L. (Celandine), and *Platystemon californicus* Benth.,

(*b*) seed-oils of some papavers—*Papaver rhoeas* L., *Papaver dubium* L., and *Eschscholtzia californica* Cham.,

(*c*) the pure alkaloids chelerythrine, quinidine, berberin, and those in the seed-oil of *Eschscholtzia californica*, and in the bark of *Bocconia fructescens* L.

Most of the active substances, by either subconjunctival or anterior-chamber injection, produced constriction of the pupil (see "Pharmacology"); adrenaline produced a marked dilatation.

Some conjunctival inflammation was seen 24 to 48 hours after the injection of active substances. The cornea showed a generalized opacity when the active substances were soluble in aqueous. The opacity was localized to the upper segment of the cornea when the active substance was an oil which floated as a drop to the zenith of the anterior chamber, thus indicating that the corneal opacity was a localized effect caused by substances diffusing from the oil. With inert substances, except for a faint trace of the needle track, no pathological changes were detected in the eye.

With severe reactions, the anterior chamber showed exudation and an irregular pupil, and the iris became attached to the cornea.

After several days, either the corneal opacity cleared up or a pannus with migrating blood vessels was formed upon it, or the opaque cornea was markedly proptosed.

Prolonged Rise in Ocular Tension after Repeated Subconjunctival Injections in Rabbits.—Of three litter-mate rabbits, one was injected with 0·1 ml. argemone oil subconjunctivally into one eye daily for 6 days. The other eye was kept as a control. The tension was taken daily in both eyes before the injection. It was raised from 12 to 32 mm. Hg, both above the basic level and above that in the control eye. When the injections were stopped for four days because of severe inflammation of the conjunctiva, the tension was maintained at least 14 mm. Hg above basic level. Two more injections were given followed by a rest of 5 days, and the tension rose to 20 mm. Hg above the basic level. Five more injections were given, the tension remaining well above the basic. There was then severe conjunctival and subconjunctival inflammation, the cornea was opaque, and the whole eye was enlarged and proptosed.

This rabbit was killed and the eyes examined histologically. The globes were markedly changed. Macroscopic observation of the treated eye showed a definite change in shape, mainly an elongation, suggesting a constriction of the limbal region. The cornea showed marked infiltration and formation of blood vessels containing blood, especially in its anterior part. The corneal epithelium was thickened. The iris showed perivascular oedema. The spaces of Fontana were very distended and contained a fibrillar coagulum. The tissues in this region were oedematous. In parts of the retina, the external and internal limiting membranes were destroyed, the Müller fibres poorly shown and oedema present. Oedema was also present around the vessels of the optic nerve.

The two other rabbits were similarly injected with 0·4 mg. sanguinarine on all the corresponding days. In one animal the tension was maintained from 10 to 23 mm. Hg above the basic level, in the other from about 10 to 16 mm. The histological changes were milder than in the eye injected with argemone oil.

Chelerythrine acting like Sanguinarine.—A pure sample of chelerythrine, a closely related alkaloid even more widely distributed among the papavers, produced acute rises in ocular tension in a series of rabbits, when injected intravenously 2 mg., intraperitoneally 7–10 mg., subconjunctivally 100 μg., or intra-ocularly 10–20 μg. The effects were quantitatively comparable with those of equivalent doses of sanguinarine.

Protection by Adrenaline, Ephedrine, and BAL.—The pharmacology of sanguinarine suggested a definite anti-adrenaline action. Experiments were conducted on a series of rabbits to determine if adrenaline and similar substances prevented or reduced either the rise in ocular tension or the pathological changes produced by argemone oil or sanguinarine.

It was first determined that 100 μg. adrenaline in water subconjunctivally, or 1 to 40 μg. adrenaline in oil intra-ocularly, produced dilatation of the pupil, but no change in the tension.

(*a*) In a rabbit, 100 μg. adrenaline subconjunctivally, completely prevented the rise in tension expected from 0·01 ml. argemone oil intra-ocularly into one eye. The tension was raised in the other eye that received the oil but not the adrenaline. Histological study showed widespread destruction of the tissues at the angle, spaces of Fontana, and the ciliary body in the eye that was not protected. These changes were less in the eye receiving adrenaline.

(*b*) In a rabbit, 0·02 ml. argemone oil intra-ocularly into one eye produced the usual rise in ocular tension of 20 mm. Hg above the basic level. The eye showed marked inflammatory reaction and low tension after 24 hours. On the next day 0·4 mg. adrenaline in oil was injected systemically, and after 30 minutes the other eye was injected with the same dose of argemone oil intra-ocularly. Adrenaline prevented the expected rise in tension, and much of the later inflammatory change. Systemic injections of adrenaline were continued daily for a week. This completely protected the second eye from later argemone effects, but did not reverse the changes of the argemone inflammation which preceded the use of adrenaline (Figs 5 and 6). Four other rabbits were similarly protected by systemic adrenaline.

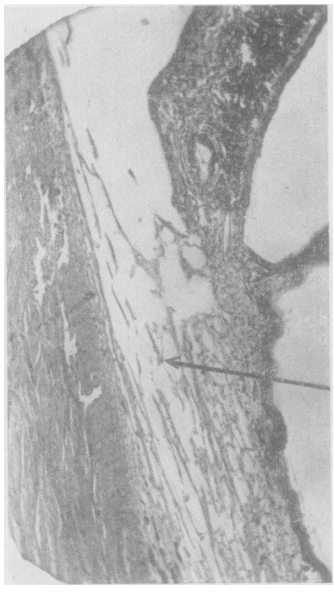

FIG. 5.—Damage to cilio-scleral spaces, and Descemet's endothelium by intra-ocular injection of 0·02 ml. argemone oil into rabbit eye.

FIG. 6.—Protection from damage caused by argemone oil, similarly introduced into the other eye, by prior and subsequent systemic injections of 0·4 mg. adrenaline in oil.

(*c*) In one eye of a rabbit, 0·01 ml. argemone oil intra-ocularly, preceded a second injection of 1 μg. adrenaline in oil intra-ocularly, by 30 minutes. Adrenaline decreased the rise in tension, but did not prevent the later inflammatory reactions. In the other eye, adrenaline was injected 30 minutes before the argemone, and the inflammatory reaction was mostly prevented.

(*d*) In one eye of a rabbit, 0·01 ml. argemone oil intra-ocularly produced a rise of 33 mm. Hg above the basic level, followed by marked reaction after 24 hours. In the other eye, an injection of a mixture of argemone oil and 1 μg. adrenaline in oil decreased the rise in tension and minimized the reaction. In another animal, a prior intra-ocular injection of 20 μg. adrenaline abolished the rise expected from 20 μg. sanguinarine.

(*e*) A systemic injection of 1 mg. ephedrine in rabbits checked the rise in tension due to argemone oil already injected intra-ocularly. Ephedrine prevented the effect of a succeeding injection of oil into the other eye.

(*f*) BAL 50 mg. systemically or 10 mg. subconjunctivally prevented the tension-raising effect of argemone oil.

(*g*) Cysteine 400 mg. systemically, 20 mg. subconjunctivally, or 2 mg. intra-ocularly prevented the tension-raising effect of argemone oil.

(*h*) Benadryl, amphetamine, priscol, glutathione, and monothioethylene glycol showed indefinite effects.

(*i*) Nembutal (pentobarbitone sodium) 60 mg. intravenously in a rabbit produced an immediate fall in the rising ocular tension caused by argemone oils but did not prevent a delayed rise.

These experiments indicate that adrenaline and ephedrine antagonize the pharmacological tension-raising effects of argemone oil and sanguinarine. Prolonged and repeated administration of the protective drugs are required to prevent the pathological changes. Adrenaline and ephedrine have been tried in epidemiological work in India.

Argemone Glaucoma and Primary Idiopathic Glaucoma.—Argemone oil is the only known substance capable of causing human glaucoma. Kirwan (1935), observing the toxic aetiology of argemone glaucoma in India, first suggested the possibility of a similar toxic aetiology in primary glaucoma in other parts of the world.

Except for particulate suspensions or gross infection, there is little evidence in the literature of any natural drug causing acute rises in ocular tension by systemic, subconjunctival, or anterior-chamber injection. The present experiments in a series of 150 rabbits have shown for the first time that both argemone oil and sanguinarine, in very minute doses, cause acute and chronic rises in tension, histo-pathological changes at the filtration angle, and retinal degeneration. Further, chelerythrine, a closely related alkaloid, several other common papaver oils like those of *Papaver dubium* and *P. rhoeas*, and extracts of some papaver plants, also produced a similar rise and changes.

The wide distribution of these papaver substances and their close relationship with argemone oil and sanguinarine seem to support Kirwan's theory of the toxic aetiology of primary glaucoma. That toxic glaucoma is not widespread may be due to the protective action of proteins in a well-fed population. The age-incidence of primary glaucoma may be related to a possible lowering of adrenaline levels or of its local production, leaving the unprotected ocular

tissues more liable to toxic action. The emotional instability of glaucoma patients may be an attempt to counteract such lowering.

Lloyd's Theory of Ingestion of Natural Papaver Substances.—Kirwan's theory has been followed up by Lloyd (1950, 51) who has shown some evidence of skin oedema and clinical similarities between argemone and primary non-congestive glaucoma. This has led him to suspect the possibility of the ingestion of common papaveraceous weeds, growing wild in England and other parts of the world. He believes that such ingestion could arise indirectly through eggs or pork. The possible contamination of cereals by such weeds, as with " tail corn " fed to domestic fowls, may lead to the indirect ingestion of their poisonous principles. Mr. Lloyd has co-operated in several of my experiments in which the activity of certain British papaver substances was demonstrated.

Diet and Endemic Glaucoma in Indian Communities.—In tracing the possible animal vectors which may transmit poisonous glaucoma-inducing substances to human populations, it must be noted that the members of the large Jain community of western India during the last 2,000 years have completely omitted meat and eggs from their diet throughout life, yet glaucoma is quite common among them. They consume large quantities of milk, and both *Argemone mexicana* L. and *Fumaria parviflora* Lam. are common weeds in their cattle-grazing areas.

The highest total incidence of glaucoma in Bombay is among the Jews, who take a high-protein meat diet; among the Muslims there is a medium incidence of glaucoma; both these communities omit pig-flesh from their diet. The Jews may be more prone to special inherited endothelial defects, such as are seen in Buerger's disease.

The emphasis on the suggestion of poisoned milk, rather than on flesh, as a vector in India, does not exclude other possibilities elsewhere.

Summary

(1) *Argemone mexicana L.*, an American weed, was introduced into the Eastern tropics in the early 18th century.

(2) Epidemics of dropsy and glaucoma in India were traced in 1926 to argemone seed-oil contaminating cooking oils.

(3) Sanguinarine, one of the active alkaloids in the oil, was isolated in 1948.

(4) The present experimental study of the oil and its alkaloid has enabled both oedema and glaucoma-like changes to be induced in animals for the first time.

(5) Argemone oil and sanguinarine appear to have three fundamental actions:

(*a*) Anti-adrenaline action.
(*b*) Histo-pathological effects on cornea, filtration angle, retina, and skin.
(*c*) Effects on tissue-fluid balance.

A single unifying " biochemical lesion " that may co-ordinate all these has not yet been found.

(6) Modifications of the actions of sanguinarine by adrenaline, food protein, and BAL may suggest therapeutic measures for epidemic and general use.

(7) The technique of oedema production by protein depletion may be employed as a new biological test for epidemiological work in the tropics, for the rapid production of toxic lesions in animals, and for the detection of other oedema- or glaucoma-producing substances.

(8) The technique of producing acute rises of tension by subconjunctival or intra-ocular injections, is a new, delicate, and rapid method of detecting tension-raising substances.

(9) The technique of causing sustained rises of ocular tension by the use of a natural plant substance, has produced pathological changes in animals similar to those of primary glaucoma. This may prove valuable for general glaucoma research.

(10) Chemical, pharmacological, and ophthalmological evidence, lends support to a theory of the toxic aetiology of primary glaucoma in other parts of the world by indirect ingestion of papaver-like substances.

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